

Combining gene expression and mutation profiling in a single cfDNA assay through the addition of a custom hybrid capture panel targeting gene regulatory regions to a commercial CGP panel 5314



Gregory M. Mayhew¹, Jonathan H. Shepherd¹, Yoichiro Shibata¹, Jeff Burdine¹, Gabriel V. Milburn¹, Kirk L. Pappan¹, Nripesh Prasad²,

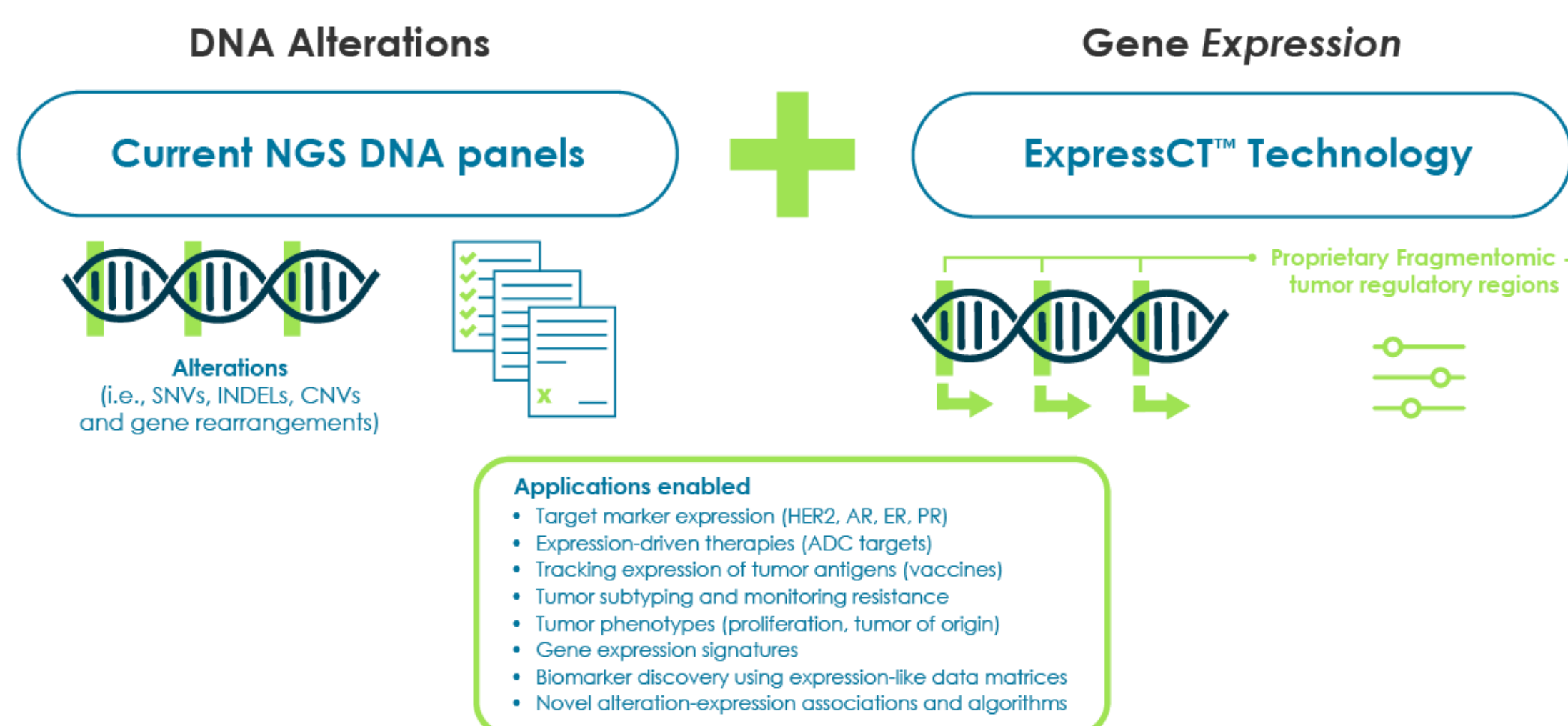
Michael V. Milburn¹, James M. Davison¹, Kirk Beebe¹

¹GeneCentric Therapeutics, Inc., Durham, NC, ²Discovery Life Sciences, Huntsville, AL

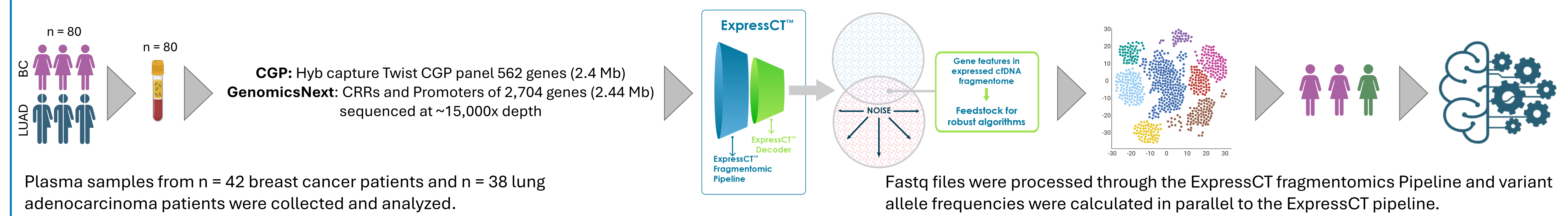


Background and GenomicsNext

Analysis of cell-free DNA (cfDNA) provides a rapid, repeatable, and non-invasive window into tumor biology, yet tissue biopsies are still often needed for accurate phenotyping and treatment guidance. A liquid biopsy approach that captures both genomic alterations and expression-like features could strengthen patient monitoring and lessen biopsy reliance. Here, we integrate a custom hybrid-capture panel targeting gene regulatory regions with a comprehensive genomic profiling (CGP) panel into a single cfDNA sequencing assay. Our bioinformatic pipeline calculates variant allele frequencies (VAF) and extracts fragmentomic features that reproducibly correlate with gene expression, enabling non-invasive tumor phenotyping.

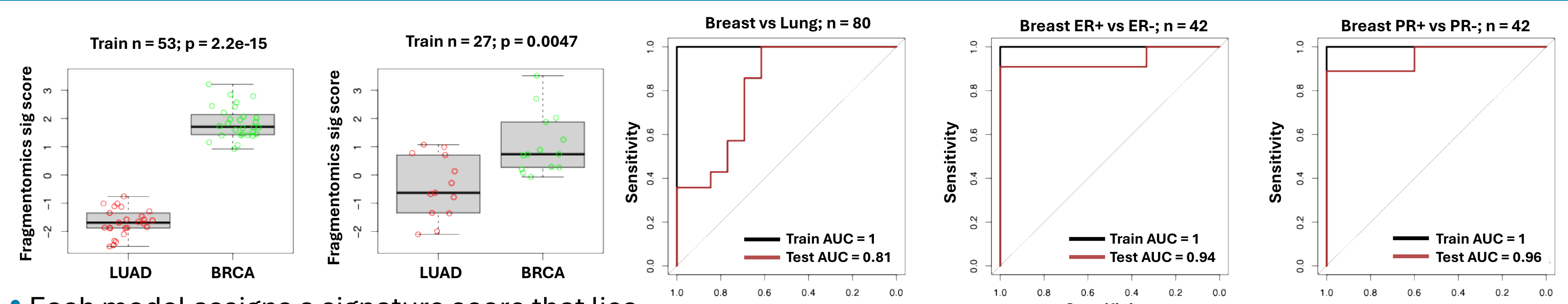


Study Design and Procedures



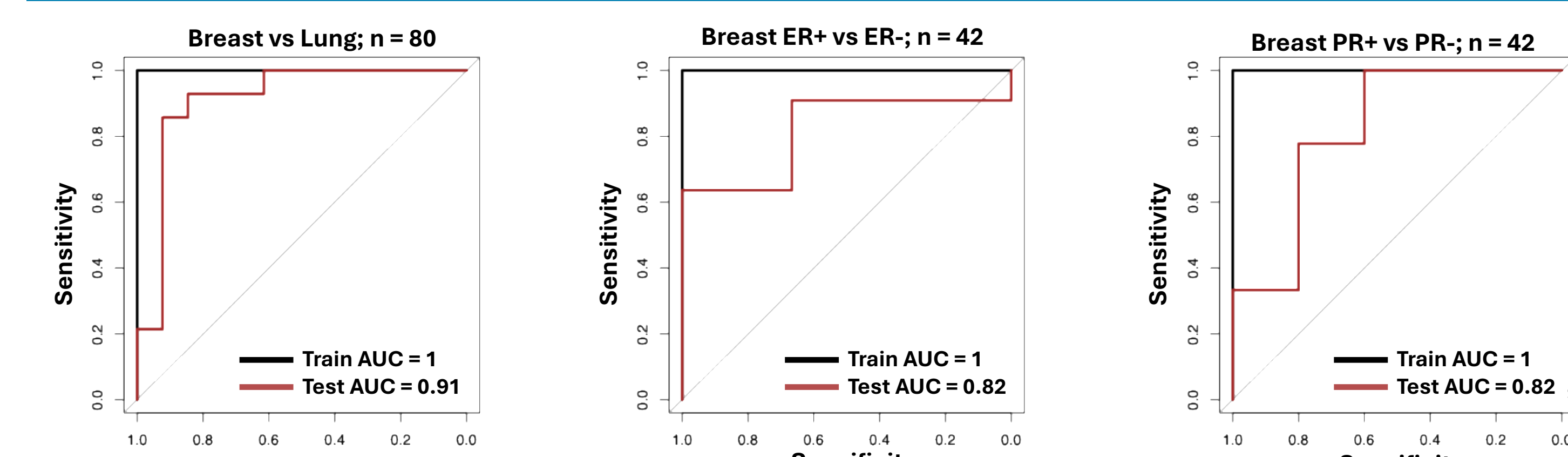
- Samples were split into train and test sets for each model and cross validation feature selection was performed; all features mapping to ChrX were excluded in Lung vs BRCA classifier.
- Classifier models were trained using features from the CGP only, the GenomicsNext panel only, and using features from both the CGP panel and GenomicsNext panel.

CGP panel Performance



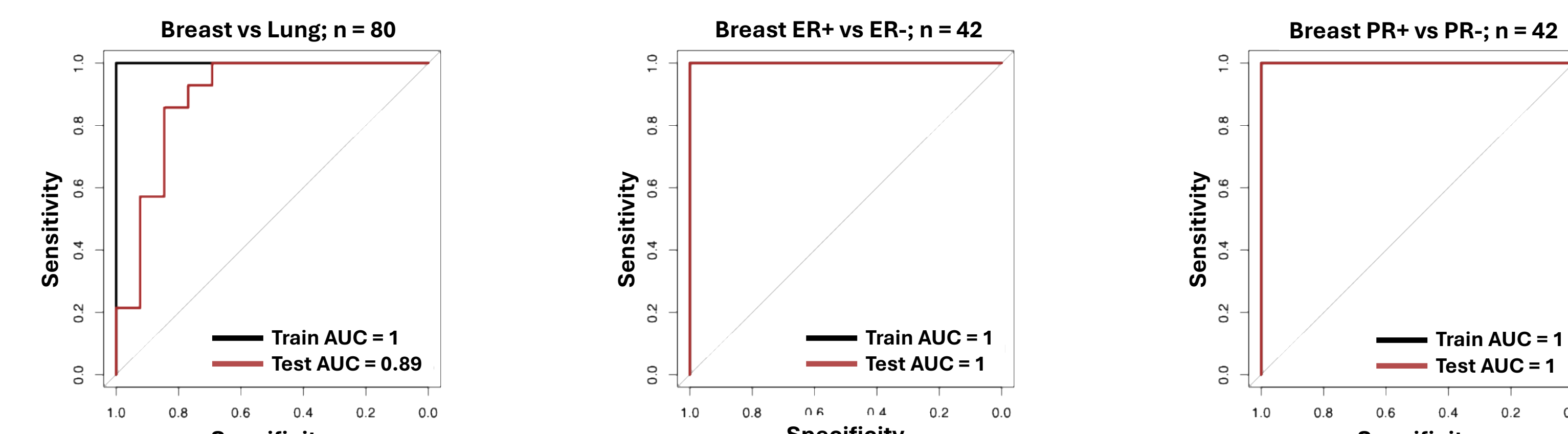
- Each model assigns a signature score that lies on a continuum and represents the strength of the call for a given subtype.
- ER and PR Models trained us CGP panel features only were highly accurate with an AUC of 0.94 and 0.96, respectively

GenomicsNext (GN) Performance



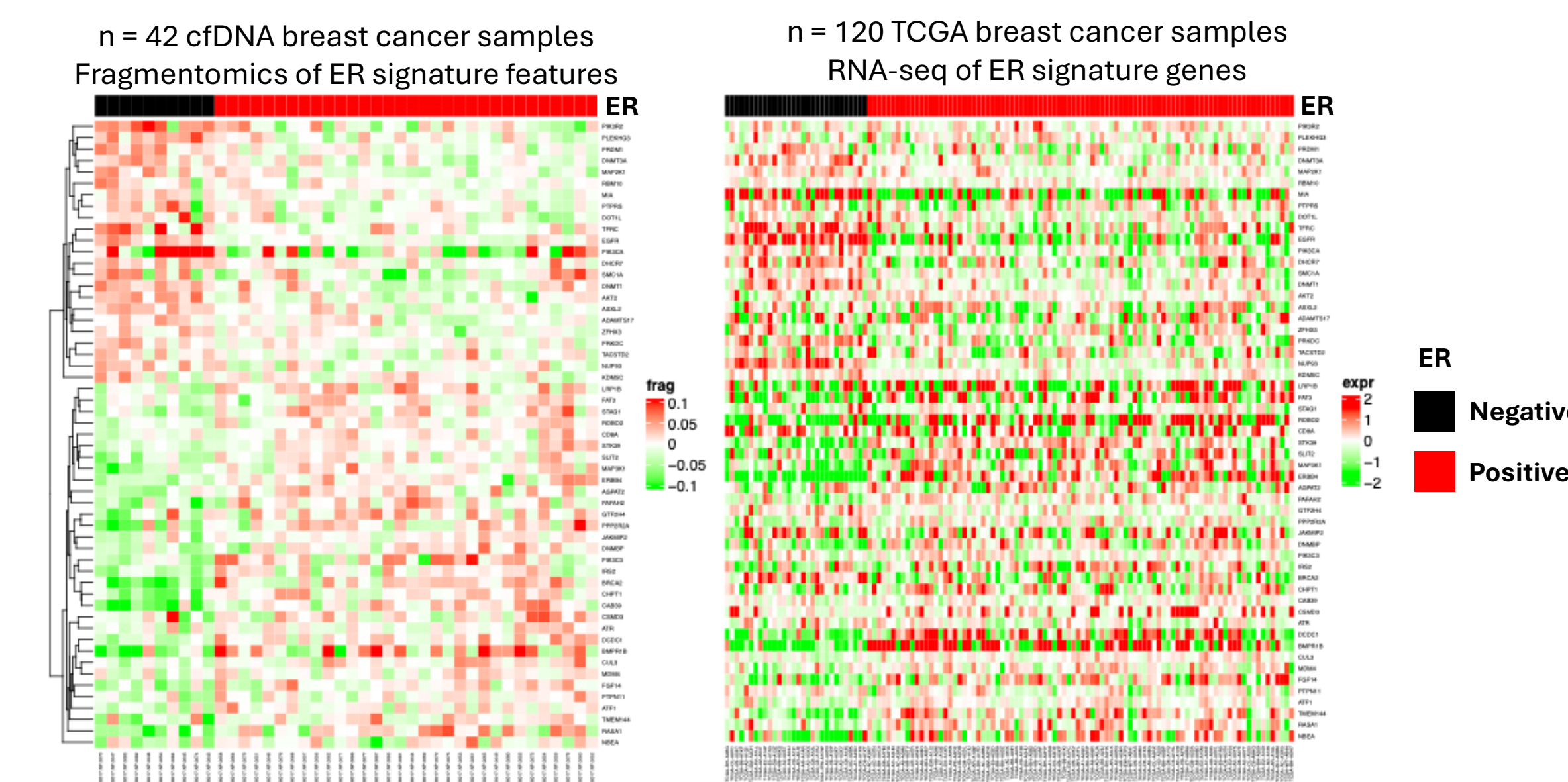
- The Breast vs Lung model trained using the GN panel features was more accurate than the CGP model; ER and PR GN models were less accurate

Combined Performance

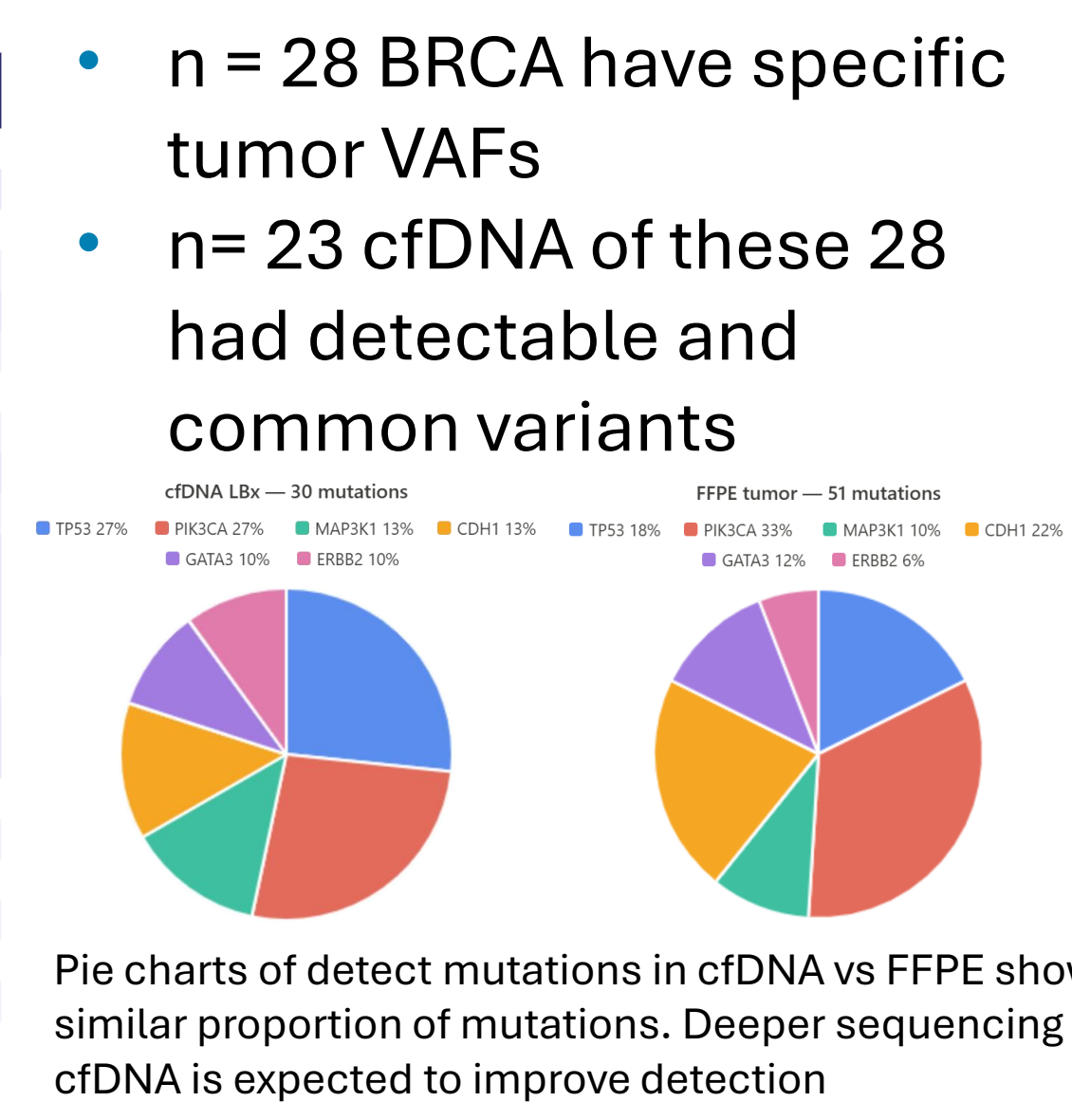


- Models trained using both CGP and GN features had the highest performance
- Individual features selected in the ER model exhibited a comparable signal profile to tumor gene expression in a separate BC cohort (right heatmaps)

Alteration Analysis



Sample ID	cfDNA variants	Ratio	Ratio	Tumor variants	Tumor variants	cfDNA variants
0017-100-0015	3	0.8052	0.8052	11	9	10.53
0017-100-0044	0	0.4086	0.5794	0	0	0.41
0017-100-0043	3	0.2060	2.1050	5	4	21.21
0017-100-0047	4	0.4086	0.2779	0	7	21.21
0017-100-0074	12	0.4086	0.9017	14	12	23.71
0017-100-0012	5	0.0080	0.2917	11	10	10.29
0017-100-0046	3	0.8052	0.1117	13	13	10.73
0017-100-0071	3	0.8750	0.2902	9	9	13.33
0017-100-0074	12	0.8544	0.4099	22	13	3.51
0017-100-0039	5	0.8074	0.4680	12	10	21.90
0017-100-0072	9	0.8176	0.6406	13	11	22.34
0017-100-0074	12	0.8160	0.2913	17	14	16.24
0017-100-0012	6	0.5460	0.4076	12	12	8.82
0017-100-0055	7	0.4144	0.2716	16	14	7.14
0017-100-0044	12	0.8544	0.3274	13	12	10.09
0017-100-0044	0	0.6664	0.4529	12	9	5.36
0017-100-0044	5	0.8267	0.5000	15	13	20.81
0017-100-0017	6	0.5460	0.4200	11	10	16.61
0017-100-0015	7	0.4144	0.2716	16	14	7.14
0017-100-0044	2	0.3910	0.5428	6	5	12.75
0017-100-0053	1	0.1950	0	5	5	10.00
0017-100-0044	1	0.2033	0	1	2.4	0.00
0017-100-0012	1	0.3300	0	1	1	10.00



Conclusions

- n = 28 BRCA have specific tumor VAFs
- n = 23 cfDNA of these 28 had detectable and common variants
- The GenomicsNext assay combined with the ExpressCT™ technology enables gene expression quantitation and DNA alterations from a single sample.
- Data support accurate tumor type classification (breast vs lung) and clinically relevant breast cancer subtyping (ER, PR).
- Performance is comparable using standard CGP panel regions or custom regulatory region panels – and strongest when combined.
- These results demonstrate a scalable path to adding expression-based biomarkers to existing liquid biopsy workflows.